

## APPLICATION OF ELECTROCHEMICAL ACTIVATED SOLUTION ON BROCCOLI SEEDS

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**Abstract.** Electrochemical activated solution (EAS) possesses a wide variety of antimicrobial activities. EAS has been known as a super disinfectant solution with high ability to kill most bacteria and fungi and is safe for humans. Therefore, it has been studied and used in many different areas of life such as medication, food processing industry, etc. However, there are few reports on the effect of EAS in agriculture. This study was conducted to determine the effects of using EAS to treat broccoli seeds on seed germination rate and growth of sprouts. The EAS was generated from KCl solution, which was then diluted with distilled water at 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 strengths of the source EAS. The results showed that treating seeds with EAS could reduce the quantity of coliform on the surface of broccoli seeds, without affecting seed germination rate and sprout growth. The 0.3 strength EAS (pH 6.7; oxidants concentration of 8.6 ppm; ORP 560 mV) was the most suitable solution for killing coliform on broccoli seeds.

**Keywords:** seed, sprout, broccoli, electrochemical activated solution.

**Classification numbers:** 1.3.1, 3.6.2.

### 1. INTRODUCTION

In recent times, people tend to switch to healthy living and healthier foods, which increases their consumption of raw sprouts. Raw sprouts are very popular in salads and many other dishes in many parts of the world, especially in Viet Nam. Sprouts are low in calories and fat, and provide substantial amounts of key nutrients, such as vitamins, minerals, proteins, enzymes, folate, and fiber [1]. Despite being a popular healthy food, multiple outbreaks linked to the consumption of raw sprouts have occurred. Most sources of these outbreaks have been traced to seeds contaminated with *Salmonella* and *Escherichia coli* O157:H7, followed by *Listeriamonocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, and *Aeromonashydrophilia* [2 - 9].

The largest outbreak linked to bean sprouts contaminated with *Salmonella* occurred in Ontario, Canada [10], and resulted in more than 600 cases of illness. In 2007, a study was conducted a region-wide assessment of the microbiological quality of retailed mung bean sprouts in the Philippines. Ninety-four percent samples were tested, and it was positive for the presence of *Salmonella* spp. and some samples had Coliform counts as high as 5.90 log<sub>10</sub>CFU/g, while *Escherichia coli* counts reached 5.50 log<sub>10</sub>CFU/g [11]. Tournas [12]

conducted a survey of fresh and minimally processed vegetables and sprouts in the Washington DC area and found that yeasts were the most prevalent organisms in these samples. Levels of yeasts can range from less than 100 to  $4.0 \times 10^8$  CFU/g, mold counts generally ranged from less than 100 to  $4.0 \times 10^4$  CFU/g [12].

One of the major causes make sprouts infected to microorganism is due to the seed used. As with many other crops, the seeds used for sprouting are obtained from plants grown in open fields without special measures, with subsequent commercial seed sprouting conditions favoring microbial growth, including that of pathogens [13, 14]. Therefore, in sprout production, assuring the absence of pathogens on seeds is regarded as a critical control point, as defined by the Codex Alimentarius Commission [15].

There are many seed decontamination methods that have been investigated over the years [16, 17]. These include chemical treatments (single chemical compound and/or combination of several chemicals) [18 - 21]. These methods gave good sterilizing efficiency but they created a quantity of chemicals released into the environment. It is reported in a study that using chemicals (HP/Carvacrol) to treat seeds reduced the germination percentage to unacceptable levels [21]. Dry-heat treatment in combination with irradiation treatment has been studied by Bari *et al.* [2], they found out that dry heat in combination with radiation doses of up to 1.0 kGy did not negatively impact the seed germination rate or length of alfalfa, broccoli, and radish seeds but did decrease the length of mung bean sprouts.

Up to now, a preeminent method to be applied in the sprouts production, which requires the reduction of microorganisms on the surface of sprouts without affecting the germination of seed, is still being sought.

EAS is usually generated by electrolysis of a saline solution in an electrochemical chamber with a diaphragm [3]. Many researches showed that EAS has the strong disinfection activities and is safe to the human and environments. It has been widely used in food processing industry [22, 23]. However, few literatures reported the effect of EAS in agriculture, especially on seed treatments. Thus, in this study, EAS was applied in the broccoli seed treatment in order to reduce the coliform on the surface of seeds, and its effect on the growth of sprouts was evaluated.

## **2. MATERIALS AND METHODS**

### **2.1. Materials**

Fresh broccoli seeds used in the experiment were obtained from VinEco Agricultural Investment, Development and Productions LLC. Seeds that are uniform in shape and size were selected for the experiment and kept at 4 °C until used. The seeds were infested with coliform at around 6 log CFU/g before experiment.

### **2.2. Experimental solutions preparation**

An EAS was generated by dissolving 20 g of KCl, as an electrolyte, in 20 L of distilled water and electrolyzing by diaphragm electrolyze with the following electrochemical conditions: 8V; 0.7A; anode flow rate 8 L/h; cathode flow rate 2 L/h. The electrochemical solution from anode and cathode were mixed together with an anode:cathode ratio of 8:0.5. The mixed solution was diluted with distilled water at 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2 and 0.1 strengths.

The pH value and Oxidation reduction potential (ORP) of EAS were measured by the HACH SenSion-156 device. The available chlorine concentration (ACC) in EAS was determined by an iodometric method (SMEWW 4500- Cl.B) and a photometric method with HACH DPD reagent (USA) on a measuring equipment DR 2800 (HACH - USA).

### 2.3. Seed treatments

In each experiment, 25 g of broccoli seeds in 250 mL beaker were soaked in the treating solutions (different EAS) for 5 min, then washed with 250 mL of distilled water.

### 2.4. Enumeration of total bacterial counts

The total bacterial counts on the broccoli seeds after being treated with different EAS and a control sample (not treated) were determined according to ISO 9308-1:2000. All plates were incubated at 37 °C for 24 h. After incubation, colonies of total natural bacteria were enumerated and expressed as colony-forming units log per gram (log CFU/g).

### 2.5. Determination of germination percentage

The germination percentage was determined as described by Hu *et al.* [24]. 5 g of samples of the control and treated seeds were placed in sterile hydroponics sponges for 3 days at an environment temperature of 25 °C ( $\pm 2$  °C), with sterile water added periodically to maintain a high-moisture environment. The total number of seeds and germinated seeds left in the containers were then counted, where the germination percentage was defined as the ratio of seeds that germinated to the total number of seeds.

### 2.6. Determination of broccoli sprouts growth

To determine the growth of broccoli sprouts, 100 sprouts of the germinated seeds in each experimental sample and control were left to continue growing for 4 days under the same cultivation conditions. The length of broccoli sprouts was measured by a ruler.

### 2.7. Statistical analysis

All trials were replicated three times under the same experimental conditions and using broccoli seeds from the same source.

Reported plate count data represent the mean values obtained from three individual trials, with each of these values being obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel 2019 program. Significant differences in plate count data were established by the smallest difference at 5 % significance level.

## 3. RESULTS AND DISCUSSION

### 3.1. Solutions properties

The pH, ACC and ORP values of different experimental solutions are shown in Table 1. Distilled water was of pH 6.9, ACC < 0.05 ppm and ORP 350 mV.

It can be seen from Table 1 that the ACC of 10 different strengths of EAS were between 2.9 - 28.6 ppm, the pH values were 6.5 - 6.7 and the ORP were 420 - 850 mV. Upon the dilution

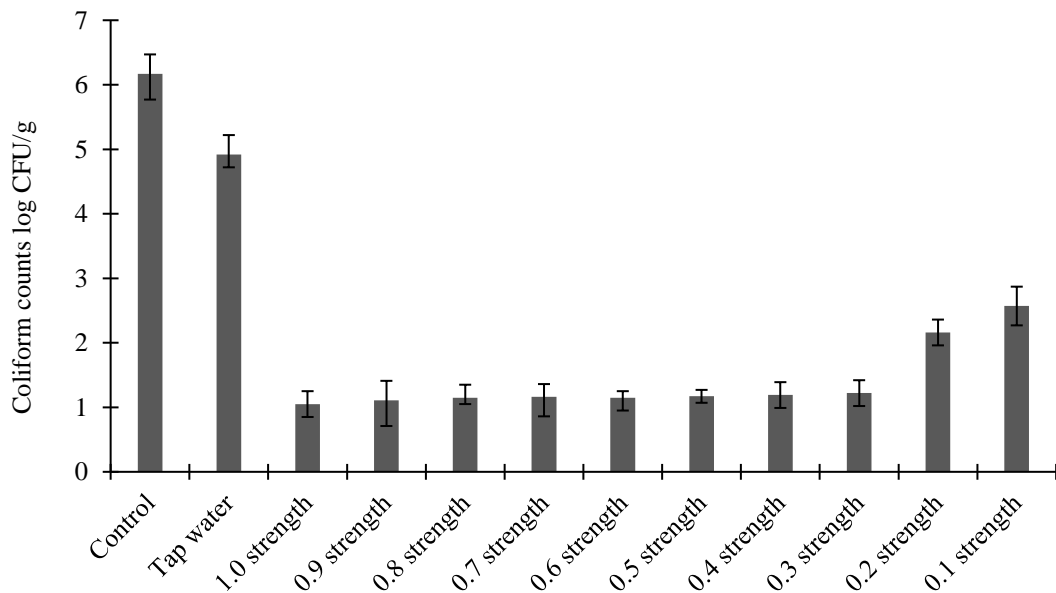
of EAS, the ACC and ORP of the diluted solution decreased, while the pH value slightly increased probably because of the reduction of ACC in the solution.

*Table 1.* The pH, ACC and ORP values of experimental solutions.

Solution	pH	ACC (ppm)	ORP (mV)
Tap water	6.7	<0.05	293
EAS 1.0 strength	6.5	28.6	850
EAS 0.9 strength	6.5	25.7	812
EAS 0.8 strength	6.5	22.9	763
EAS 0.7 strength	6.6	20	720
EAS 0.6 strength	6.6	17.2	685
EAS 0.5 strength	6.6	14.3	643
EAS 0.4 strength	6.6	11.4	607
EAS 0.3 strength	6.7	8.6	560
EAS 0.2 strength	6.7	5.7	506
EAS 0.1 strength	6.7	2.9	420

### 3.2. Effect of EAS on the total bacterial counts on the surface of broccoli seeds

The effect of EAS on the total bacterial counts on the surface of broccoli seeds is presented in Fig. 2.



*Figure 1.* The effect of EAS on the coliform counts on the broccoli seeds surface.

The coliform counts of seeds in control sample and treated with tap water reached as high as 6.17 and 4.92 log CFU/g, respectively. Meanwhile, the coliform counts on the seeds treated with EAS declined sharply to a mere 1.05 to 2.57 log CFU/g. At the available chlorine concentration of 28.6 - 8.6 ppm (EAS strength 1.0 to 0.3), the coliform counts on seeds reduced strongly (total coliform between 1.05 and 1.22 log CFU/g), but there was no considerable difference of total coliform among these samples. In the meantime, the total bacterial counts were substantially higher on the seeds treated with EAS of 0.2 and 0.1 strength (ACC of 5.7 and 2.9 ppm, correspondingly), at 2.16 and 2.57 log CFU/g, respectively.

This result showed that the sterilization capacity of EAS for broccoli seed increased with an increase in available chlorine concentration in it. However, at available chlorine concentrations of 8.6 - 28.6 ppm (EAS strength 0.3 to 1.0), the effect of EAS was not significantly different.

In comparison with other methods about positive effect, like the combinations of high-pressure treatment, temperature and antimicrobial compounds method studied by Peñas *et al.* [21], at a hypochlorite concentration of 18.000 ppm and a pressure of 200 MPa, the microbial reduction was achieved at 4.5 - 5 log CFU/g, while the EAS treatment method gave the coliform counts reduction up to 5.12 log CFU/g at an ACC concentration of a mere 28.6 ppm in normal condition. This strong disinfecting effect was due to the fact that the EAS contains not only chlorine-containing oxidants but also many other strong oxidants such as atomic oxygen, single molecular oxygen  $^1\text{O}_2$ ,  $\text{O}_3$ , free radicals, etc. These oxidants have been shown to have strong disinfecting effect even at a small concentration. In addition, the EAS always exists in a state of stable pseudo-stimulation, whereas the oxidants composition is always changing. As such, microorganisms are unable to adapt to resist [25]. Meanwhile, the small concentration of oxidants has ensured safety for human health and the environment during long-term use [26]. This EAS treatment method not only has sterilization effect as high as the combinations of high-pressure treatment, temperature and antimicrobial compounds method, but also is easier to apply and cheaper because high pressure and temperature condition is not necessary.

Another method is the dry-heat treatment in combination with irradiation treatment proposed by Bari *et al.* [2], where under the condition of dry heat for 17 h associated with irradiation at a dose of 0.25 kGy, the *E. coli* from broccoli seeds (the *E. coli* counts before treatment was 5.2 log CFU/g) was completely eliminated. This method gave higher sterilization effect than the EAS method. However, the dry-heat treatment in combination with the irradiation method requires a long treatment time (dry heating for 17 h) and consumes a lot of electricity. Therefore, the Bari's method is difficult to be widely applied because of the equipment and operating costs. On the other hand, the EAS method is much faster and easier to apply, but still can provide the necessary sterilization efficiency.

Furthermore, since the production cost of 1 liter of EAS was about only 2.000 VND (about 0.1 USD), comparing to other seed treatment methods, the cost of applying this method was much lower. In comparison with other chlorine compounds, using EAS is more efficient and safer due to the significant limitation of the formation of organic halogen compounds [27].

### 3.3. Effect of EAS on the germination percentage of broccoli seeds

The effect of EAS on the germination of broccoli seeds is shown in Fig. 2.

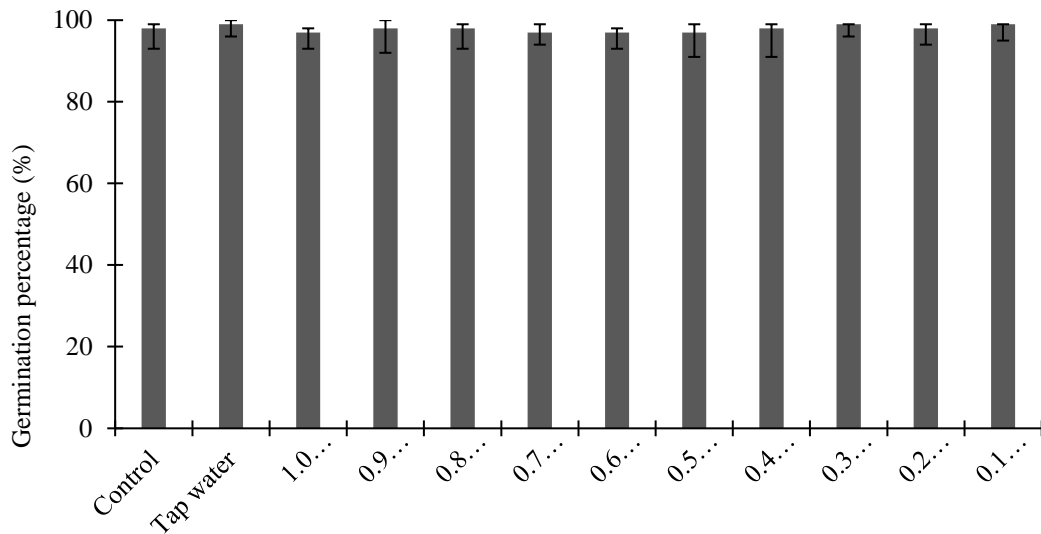


Figure 2. The effect of EAS treatment on the germination percentage of broccoli seeds.

After treatment with different EAS strengths and tap water the germination rate of broccoli seeds was around 96 - 99 %. There was no considerable difference in the number of germinated seeds among these experiments. This result showed that the seed treatment by EAS did not have a significant negative impact on the germination percentage of broccoli seeds.

### 3.4. Effect of EAS on the growth of broccoli sprouts

The effect of EAS on the growth of broccoli sprouts was evaluated by the total length obtained after sowing for 7 days and the results were shown in Fig. 3.

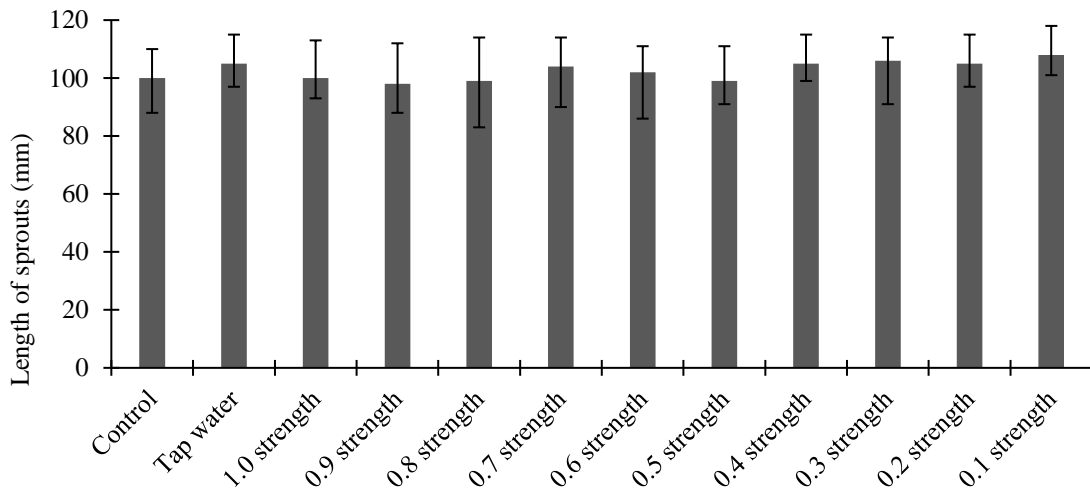


Figure 3. The effect of EAS treatment on the growth of broccoli sprouts after sowing for 7 days.

For the control sample (no treatment) and treatment with tap water, the average length of broccoli sprouts was around 100 and 105 mm, respectively. The broccoli sprouts sowed with the

treated seed at different strengths of EAS had an average length of 97 to 108 mm. However, the growth differences of broccoli sprouts were not obvious among the treatments. This showed that the seed treatment with EAS (at pH 6.5 - 6.7; ACC 2.9 - 28.6 ppm) had no impact on the growth of broccoli sprouts.

#### 4. CONCLUSIONS

The electrochemical activated solutions with different strengths (1.0; 0.9; 0.8; 0.7; 0.6; 0.5; 0.4; 0.3; 0.2; 0.1), pH values of 6.5 - 6.7 and available chlorine concentrations of 2.9 - 28.6 ppm were successfully generated to be applied for broccoli seed treatment. The results showed that the EAS had good effect on reducing the coliform on the broccoli seed surface, without affecting the germination percentage or average sprout length. The most suitable condition for the treatment of broccoli seeds was at pH 6.7 and ACC of 8.6 ppm. This EAS holds great promise for use in seed treatment because of its high coliform killing effect, lowcost, time saving and ease of application.

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**CRediT authorship contribution statement.** Nguyen Thi Thanh Hai: Investigation, Methodology, Formal analysis, Supervision. Truong Anh Dung: Methodology, Formal analysis. Nguyen Thi Quy: Formal analysis.

**Declaration of competing interest.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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